

IN THE UNITED STATE PATENT AND TRADEMARK OFFICE

In re PATENT Application of  
Saville, et. al.

Group Art Unit: 1651

U.S. Serial No. 10/797,019

Examiner: Gough, Tiffany Maureen

Filed: 11 March 2004

Att. Docket No.: 95773-1439

For: **ENHANCEMENT OF ENZYME ACTIVITY BY SELECTIVE PURIFICATION**

4 August 2010

APPEAL BRIEF

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

This is an appeal from the final rejection of claims 2-7, 11-14, 31-36, 39, 40, 42-64, and 66 of the subject application.

(i). Real Party in Interest:

This application is owned by Immortazyme Company.

(ii). Related Appeals and Interferences:

There are **no** other prior or pending appeals, interferences or judicial proceedings known to Appellant, Appellant's legal representative, or assignee which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(iii). Status of Claims:

Claims 2-7, 11-14, 31-36, 39, 40, 42-64, and 66 are pending in this application.

Claims 2-7, 11-14, 31-36, 39, 40, 42-64, and 66 stand rejected.

The rejection of claims 2-7, 11-14, 31-36, 39, 40, 42-64, and 66 is appealed.

Please see the Claims Appendix for a copy of the claims under appeal. The claims under appeal include the amendments made in the after final rejection filed on 3 June 2010.

(iv). Status of any Amendment Filed Subsequent to Final Rejection:

An amendment after final rejection was filed on 3 June 2010, which was entered for purposes of appeal in the Advisory Action mailed 23 June 2010.

A Notice of Appeal was filed on 5 May 2010 along with the appropriate fee and petition for extension of time.

(v). Summary of Claimed Subject Matter:

Independent claim 31 provides a method of enhancing the intrinsic enzymatic activity of an enzyme formed from fermentation comprising:

- (a) diluting an enzyme solution comprising glucoamylase with an aqueous solution by a factor of at least three to provide a diluted enzyme solution;
- (b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;
- (c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and
- (d) removing the activated carbon to provide an enzyme solution of enhanced activity. Basis for claim 31 can be found in the originally filed application including at page 2, lines 22-36; page 3, lines 26-2; and page 4, lines 1-18.

Independent claim 42 provides an enzyme solution having enhanced activity made by a method comprising:

- (a) diluting an enzyme solution comprising at least one of glucoamylase or amylase with an aqueous solution by a factor of at least three to provide a diluted enzyme solution;
- (b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;
- (c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and
- (d) removing the activated carbon to provide an enzyme solution of enhanced activity. Basis for claim 42 can be found in the originally filed application including at page 2, lines 22-36; page 3, lines 26-2; and page 4, lines 1-18.

Independent claim 46 provides a method of enhancing the intrinsic enzymatic activity of an enzyme formed from fermentation comprising:

(a) diluting an enzyme solution comprising amylase with an aqueous solution by a factor of at least three to provide a diluted enzyme solution;

(b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;

(c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and

(d) removing the activated carbon to provide an enzyme solution of enhanced activity. Basis for claim 46 can be found in the originally filed application including at page 2, lines 22-36; page 3, lines 26-2; and page 4, lines 1-18.

(vi). Grounds of Rejection to be Reviewed on Appeal:

- A. Whether claims 34, 35, 45, 61 and 62 comply with 35 U.S.C. § 112, first paragraph.
- B. Whether claims 2-7, 11-14, 31, 34, 42, and 46-64 comply with 35 U.S.C. § 112, second paragraph.
- C. Whether claims 2-7, 31-33, 39, 40, 42-44, 46-52, 63, 64 and 66 are patentable under 35 U.S.C. § 103(a) over Published U.S. Patent App'n Ser. No. 2002/0020668 (Lausten) in view of FEBS Letters, 1977 (Schuster).
- D. Whether claims 2-7, 12, 13, 31-33, 39, 40, 42-44, 46-61, 63, 64 and 66 are patentable under 35 U.S.C. § 103(a) over Lausten in view of Schuster, J. of Bioscience, Vol.7 1985 (Shenoy) and [http://22.ap-lab.com/circular\\_dichroisms.htm](http://22.ap-lab.com/circular_dichroisms.htm).
- E. Whether claims 2-7, 11, 14, 31-33, 39, 40, 42-44, 46-58, 64 and 66 are patentable under 35 U.S.C. § 103(a) over Biotechnology Letters, Vol. 23, 2001, p. 295-301 (Aikat) in view of each of Lausten and Schuster.

(vii). Argument:

**A. Claims 34, 35, 45, 61, and 62  
Comply with 35 U.S.C § 112, first paragraph.**

Claims 34 and 61 stand or fall together for purposes of this appeal.

Claims 35, 45 and 62 stand or fall together for purposes of this appeal.

Claims 34, 35, 45, 61, and 62 fully comply with 35 U.S.C. 112, first paragraph.

Claims 34 and 61 recite the specific language used in Example 1 disclosed in the originally filed application and suggested by the Examiner. The Examiner stated "Applicant should consider language as recited in example 1," which Appellant complied with. See page 3 of the 23 January 2009 Office Action. Claims 34 and 61 recite "the enzyme activity of the diluted enzyme solution is at least statistically equivalent to the enzyme activity of the enzyme

solution before dilution," which is supported by the language in Example 1 that states, "the activity of the diluted enzyme before purification (12) was statistically equivalent to that of the raw enzyme (19)." See page 5, lines 28-30 of the originally filed application. No new matter has been added.

Claims 35, 45 and 62 recite that the solution is enhanced by at least 200%. Claims 35, 45 and 62 are not rejected over prior art.

It is well known in the art that a dilution factor of 1 is no dilution (0%), a dilution factor of 2 is doubling (100%) and a dilution factor of 3 is tripling (i.e. 200%). The original specification at page 4, line 12, discloses dilution to "a factor of at least three," which one of ordinary skill in the art would understand to be a dilution of "at least 200%." The original specification also teaches that preferred dilution factors are 5-10, in which a factor of 10 is 900%. See page 4, line 4. Thus, the claimed end point "at least 200%" is clearly supported by the originally filed application.

Page 4, lines 15-16 of the original specification teaches that (1) the diluted enzyme has at least the same level of activity as the undiluted enzyme ("at least maintaining its original level of enzymatic activity" after being diluted). One of ordinary skill in the art is well aware that if a 200% dilution and higher dilutions have the same level of activity as the undiluted enzyme after treatment according to the present invention, then "the activity of the enzyme solution is enhanced by at least 200%." If the activity was not enhanced (increased), the enzyme would not have same level of activity after dilution. There is no other plausible interpretation of these teachings in the originally filed application. Thus, the original application provides clear support for claims 35, 45 and 62.

Appellant submits that the claims fully comply with Section 112. Accordingly, withdrawal of the Section 112, first paragraph, rejection is respectfully requested.

**B. Claims 2-7, 11-14, 31, 34, 42 and 46-64  
Comply with 35 U.S.C § 112, second paragraph.**

Claims 2-7, 11-14, 31, 34, 42 and 46-65 fully comply with 35 U.S.C. § 112, second paragraph.

The term "enzyme weight" is clearly defined on page 3, lines 23-30 of the originally filed specification as follows:

By the term "raw enzyme in connection with its weight" as used in this specification and claims is meant the volume of the raw enzyme solution x the density of the raw enzyme solution.

The weight ratio of raw enzyme to purifying agent is dependent on the enzyme and purifying agent. Preferably, the ratio is not greater than 50:1, more preferably, not greater than 25:1, and still more preferably not greater than 15:1. A preferred ratio for use with activated carbon as the purifying agent provides 11g raw enzyme purified with 0.75 g activated carbon.

Thus, the claimed phrase "raw enzyme weight to activated carbon weight ratio" is clearly defined in the specification and fully complies with Section 112. Accordingly, withdrawal of the Section 112, second paragraph, rejection is respectfully requested.

**C. Claims 2-7, 31-33, 39, 40, 42-44, 46-52, 63, 64, and 66 are  
patentable under 35 U.S.C. § 103(a) over Lausten in view of Schuster**

Claims 2-7, 31-33, 39, 40, 46-52, 63, 64 and 66 recite **methods**, which stand or fall together for purposes of this appeal.

Claims 42-44 recite **products**, which stand or fall together

Claims 2-7, 31-33, 39, 40, 42-44, 46-52, 63, 64 and 66 are patentable under 35 U.S.C. § 103(a) over Lausten in view of Schuster.

**The methods recited in claims 2-7, 31-33, 39, 40, 46-52, 63, 64 and 66 are patentable over the combination of Lausten and Schuster for the following reasons.**

The methods recited in claims 2-7, 31-33, 39, 40, 46-52, 63, 64 and 66 relate to enhancing the intrinsic enzymatic activity of an enzyme formed from fermentation comprising:

(a) diluting an enzyme solution comprising glucoamylase or amylase with an aqueous solution by a factor of at least three (200%) to provide a diluted enzyme solution;

(b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;

(c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and

(d) removing the activated carbon to provide an enzyme solution of enhanced activity.

The present invention is distinct from Laustsen because the present invention requires the combination of:

1. Claimed invention removes cells if present (purification in step b) and Laustsen does not;
2. Claimed invention has a far higher level of dilution (at least three-fold, and preferably 5-fold or 10-fold), while Laustsen cites, at most, a 1.1-fold dilution; and
3. Claimed invention has a high ratio of activated carbon to enzyme (50:1) to provide the claimed enhancement of enzyme activity.

Appellant notes that Laustsen discloses in paragraph 46 that "[a]ccording to the present invention the added amount of carbon is preferably from 0.05 to 2% (w/w) of the initial fermentation broth volume, in particular the added amount of carbon is from 0.1 to 1% (w/w) of the initial fermentation broth volume." At the maximum of 2%, this is a 50:1 range of fermentation broth to activated carbon. However, Laustsen clearly teaches to use amounts of activated carbon in a direction away from the claimed invention, i.e. far



less activated carbon. First, Laustsen's preferred range is 0.1 to 1%, which at 1% is a 100:1 ratio. Second, all of Laustsen's working Examples 1-3 teach far less activated carbon, i.e., 0.2%, which is a 500:1 ratio. Furthermore, the Rule 132 Declarations of record demonstrate that even at low activated carbon amounts, 500:1, the process of Laustsen did not work unless pressure was applied and, thus, the much higher amounts of carbon claimed (not greater than 50:1) would cause further problems in the process of Laustsen, as discussed in the Rule 132 Declarations and below. In contrast, in the claimed invention the ratio of broth to carbon is not greater than 50:1. Note that a ratio of 49:1 has more activated carbon than a ratio of 50:1. Appellant further points out that the claimed activated carbon ratios are used in combination with other claimed steps, such as at least a 3 fold (200%) dilution and filtering the cells before contacting with activated carbon for a time sufficient to effect enzyme enhancement, which combination is not taught by Laustsen.

Laustsen teaches a purification method that results in a 12% increase in flux. See page 2 of Laustsen's 2 December 2002 Response, copy submitted by Appellant. Laustsen does not provide any disclosure or even a suggestion of how to improve enzyme activity so that less amounts of enzyme are necessary.

Schuster does not provide the deficiencies of Laustsen. Schuster also does not observe the effect of activated carbon on the activity of the purified enzyme solution. In fact, a necessary condition of Schuster's observations is the presence of tightly bound ligands such that the treatment with activated carbon can remove these tightly bound ligands allowing the enzyme to attack the substrate uninhibited. Since there are very few, if any, inhibitors in the 'raw enzyme solution', which is a partially purified enzyme solution, one skilled in the art would not anticipate that the activated carbon would have any effect. While Schuster treats purified P450 with activated carbon, there is no attempt to quantify any increase in activity of the enzyme after such treatment. Schuster merely tests the ability of the activated carbon to release an antibiotic that has been added to the purified protein *in vitro*. This is very different from the present invention where a partially purified raw enzyme solution has been filtered and clarified

(page 2 lines 29 to 34) to obtain an enzyme solution devoid of endogenous inhibitors. In light of Schuster, one skilled in the art would believe that, in the absence of any inhibitors or other tightly bound ligands, activated carbon would have no positive effect. Thus, the combination of Lausten and Schuster cannot teach or suggest the claimed method of enhancing enzyme activity. For this reason alone, the Section 103 rejection should be withdrawn.

Even if the cited references were combined, the experimental evidence of record rebuts any prima facie case of obviousness based on Laustsen, or any other reference that only teaches purification. The experimental evidence now of record demonstrates that the claimed invention results in a surprising 200 to over 900% increase in enzyme activity.

The experimental evidence disclosed in the originally filed patent application, the April 26, 2007 Rule 132 declaration, the 13 March 2008 Rule 132 declaration, and the numerous articles of record, Appellant has conclusively demonstrated that:

- 1) Merely contacting undiluted commercial enzyme through activated carbon provides no measurable increase in enzyme activity.
- 2) Raw fermentation broth containing cells diluted by ~55% (approximately 1:1, the maximum taught by Laustsen) cannot be processed through activated carbon by the claimed method. The negligible permeability is due to the presence of cells in the system, Appellant's use of high levels of activated carbon, and the fact that Appellant's process can be practiced under ambient pressure.
- 3) Raw fermentation broth which has cells removed and has been substantially diluted, e.g., 10-fold, or 1 part broth with 9 parts water/buffer, *can* be processed through the large quantity of activated carbon specified in the present patent application.

- 4) Commercial enzyme that has simply been diluted is well known in the art to have reduced activity, which is further confirmed by the experimental results in the rule 132 declarations. In contrast, processing the diluted enzyme through activated carbon (as specified in the present patent application) leads to an unexpected significant (multi-fold) enhancement of activity.
- 5) Purification, as understood by those skilled in the art, does NOT automatically lead to an increase in enzyme activity. As demonstrated by the numerous references of record, a reduction in activity after purification is far more likely. Thus, purification methodologies are designed to minimize this well known adverse outcome
- 6) Purification, as understood by those skilled in the art, may lead to an undesirable change in structure and, thus, a change in CD or UV spectrum. This change in structure (UV and CD spectrum), as outlined in point (5), is known to be deleterious, and causes a REDUCTION in enzyme activity. Thus, conventional purification methodologies use structural analysis post-purification to ensure that structure has been PRESERVED, since this corresponds to retention of activity or minimizes the reduction of activity.
- 7) In an embodiment of the present invention, the interaction between the diluted purified enzyme and the activated carbon leads to a desirable structural change in the protein, as evidenced by a change in CD/UV spectrum and electrophoretic bands that is surprisingly not deleterious. Unlike conventional purification, this change in structure provides a heretofore unknown multi-fold INCREASE in activity. It is Appellant's hypothesis, without being bound by any theory, that this unexpected and substantial increase in activity arises from activated carbon's role as a catalyst and Appellant therefore refers to a catalytic or chemical change in protein structure, the result of which is an increase in activity. While activated

carbon is known to have catalytic activity, it was unknown prior to the present invention that activated carbon could catalytically increase the activity of a purified and diluted enzyme, and surely not to such a large degree, up to 1,000%.

The starting materials used in Example 2 of Laustsen are not all commercially available. Thus, Appellant contracted a fermentation facility to produce Laustsens' alpha amylase in accordance with the British patent 1,296,839 cited by Laustsen, and then attempted to process the raw fermentation broth according to the conditions and ratios described by Laustsen in Example 2. For comparison, Appellant then also performed experiments using the fermentation broth diluted to 10% of its original concentration (a dilution ratio according to the present invention), and Appellant also performed experiments with an essentially cell-free enzyme preparation, following the claimed invention. Experiments were performed with the low level of activated carbon specified by Laustsen, and with the high level of activated carbon specified in claimed invention. Details of the experiments and the experimental observations are described in the Rule 132 Declarations.

Appellant's original plan was to test the activity of the enzyme processes according to the procedure described by Laustsen. However, when Appellant ran the process according to Laustsen, none of the enzyme eluted, even though a more porous filter was used, so Appellant did not have a sample to test. Increasing the amount of activated carbon, as per the present invention, did not improve the situation. Appellant was only able to collect samples from trials where the conditions were dramatically modified from those described by Laustsen, e.g., (1) by using much more diluted fermentation broth (1 part broth in 9 parts water, versus 1 part broth plus ~1 part water, as per Laustsen), (2) removing the cells first, as per the present invention, or (3) using a highly porous filter cloth. Even in the case of method (3), once the cells accumulated on the surface, Appellant could not collect much enzyme from the broth. Ultimately, these experiments confirmed that the claimed process is dramatically different from that

described by Laustsen, and that cell removal and significant pre-dilution are key prerequisites to using the claimed process.

Furthermore, Appellant has demonstrated that large quantities of activated carbon are a disadvantage when the goal is filtration (Laustsen), but Appellant's own work has also shown that large quantities of activated carbon are essential if the goal is activity enhancement. This further distinguishes the claimed method from Laustsen's. Since Laustsen's stated goal is filtration improvement, Laustsen cannot teach the use of large quantities of carbon, because it adversely affects filtration rate (flux). Without sufficient carbon (or retention time in the carbon), it is difficult to detect any change in enzyme activity if any actually occurs. Conversely, the present invention must use high levels of activated carbon, otherwise, Appellant does not get the claimed enhancement of enzyme activity. Too little activated carbon is a disadvantage for the present invention, whereas too much activated carbon is a disadvantage for Laustsen and, thus, Laustsen teaches in a direction away from the claimed invention. Given the claimed invention's distinct objectives, the operating conditions, dilution levels, and carbon loading must be distinctly different from the process of Laustsen.

Laustsen is owned by Novozymes, which is one of largest producers of enzymes in the world. Thus, Novozymes is highly skilled in the art of producing enzymes. From an economic perspective, given the significant shortage of fermentation capacity in the U.S., and the high cost to produce and ship enzymes, it would be enormously surprising if Novozymes failed to adopt the presently claimed technology if they found an activity enhancement from their work with the Laustsen application. The present invention conservatively reduces enzyme costs by 10 to over 90% in a multi-billion dollar industry. Certainly, Laustsen, as a Novozymes employee, would therefore have primarily emphasized and claimed any results of enhanced activity, rather than results of enhanced permeation, if enhanced activity had been observed. Instead, the obvious conclusion is that enhanced activity was neither detected, nor expected by Laustsen. As noted above, to achieve their goal of enhanced microfiltration, Laustsen has to use small amounts of activated carbon to avoid clogging the filter, insufficient to create the

activity enhancement seen (or possible) with the activated carbon loadings used in the presently claimed process. Thus, Laustsen actually teaches in a direction away from the claimed invention.

More specifically, the Rule 132 Declarations confirm that:

(1) A raw fermentation broth, even when diluted by ~55% as specified by Laustsen, cannot flow through the column. Even with very low quantities of activated carbon (i.e., 0.3g per 310 mL of diluted fermentation broth (1050:1 ratio)), there is no permeation through the column and column frit even though the frit utilized had a pore size of 20 $\mu$ m, which was far larger than Laustsen's disclosed pore size of 0.45 $\mu$ m. Thus, Laustsen's microfiltration process is dramatically different from the claimed method, which uses the combination of contact with high levels of activated carbon for a time sufficient to effect enzyme enhancement, much higher dilutions, and an essentially cell-free solution. Furthermore, Laustsen applies pressure across the membrane in order to achieve the stated fluxes, whereas the claimed invention is not limited to any particular pressure.

(2) If the porosity restriction imposed by the column frit is removed in Laustsen, e.g., by replacing the column frit with filter cloth, some very limited permeation of the diluted fermentation broth is observed, but the rate drops off rapidly once the cells accumulate within the activated carbon. Operating with the high level of activated carbon specified in the present application restricts the flow even further. Thus, the fermentation broths discussed by Laustsen cannot be processed using the level of activated carbon specified by the present invention, even if the fermentation broth is diluted by ~55% as specified by Laustsen. If the broth is diluted to 10 times its original volume (a dilution rate specified by present invention), permeation is more rapid for a while, until the cells collect on the filter cloth, after which the permeation slows dramatically again.

(3) If a cell-free enzyme solution is used according to the claimed invention, the resulting diluted enzyme is readily permeable through the column, whether with the original column frit or with the filter cloth. If the filter cloth is used, the diluted enzyme passes through the column in a matter of seconds. This shows the need for a nearly

cell-free solution and the need for a significantly diluted enzyme in order to process through the amount of activated carbon specified by claimed invention.

Also provided in the Rule 132 Declarations are the test results of a large scale production run using several hundred litres of a diluted enzyme solution produced according to the present invention. This work, conducted in 2003/04 in a 20 million gallon per year ethanol plant, demonstrates that the claimed modified alpha amylases surprisingly matched the performance of the industry standard alpha amylase (Liquozyme, from Novozymes) when added to the liquefaction stage of the ethanol plant. These experiments were based on an 80%/20% blend of the presently claimed modified enzymes with Liquozyme. The resulting sugar profiles, fermentation profiles, and flow data showed that the resulting blend was bioequivalent to the 100% Liquozyme, **in spite of the fact that the blend contained 75% less raw alpha amylase**. Clearly, the claimed process was instrumental in dramatically increasing the activity of the alpha amylase. Based on the specified addition rate of 65 mL/min, a 75% reduction in alpha amylase translates into a volumetric savings of around 2100 Litres per month, and thousands of dollars in savings to the ethanol plant.

The Rule 132 Declarations also provide a comparison between the mere dilution of a purified commercial enzyme compared to a diluted purified commercial enzyme that has been processed according to the claimed invention to provide enhanced enzyme activity. The experimental results conclusively demonstrate that the diluted enzyme processed according the present invention exhibited a surprisingly far greater activity than the merely diluted enzyme.

The experimental evidence of record, both in the originally filed application and the Rule 132 Declarations of record, fully rebut any prima facie case of obviousness raised by the Examiner. Accordingly, withdrawal of the Section 103 rejection is respectfully requested.

**Appellant Addresses Below Each Argument  
Raised by the Examiner in the Final Office Action.**

On pages 11-12 of the Final Office Action, the Examiner argues that:

It is the Office's position that it is well known by one of ordinary skill in the art that diluting and using a purifying agent such as activated carbon would enhance enzyme activity by removing endogenous inhibitors. It is well known that purifying agents such as activated carbon are adsorptive, remove inhibitors and are used for purifying compounds, especially enzymes. Applicant argues that the Declaration submitted demonstrates that the claimed invention results in a surprising 200-900% increase in enzyme activity. Applicant's declaration does not appear to be commensurate in scope with the claimed invention or what is disclosed in applicant's specification. Applicant is comparing the enzymes, Liquezyme and Allzyme, and their effects on ethanol production and glucose conversion. Applicant's third comparison is similar to the second, however, comparing the effects of diluting glucoamylase and its effects on maltodextrin conversion. These comparisons are not commensurate in scope with the claimed invention and introduce confusion as to what "activity" is actually being increased. Is the actual measured activity measured in U/mL or is it the enzyme's ability to convert starches and how are those activities intrinsic?

Appellant respectfully submits that the Examiner has not provided any reference citations to support the Office's position that "diluting and using a purifying agent such as activated carbon would enhance enzyme activity by removing endogenous inhibitors." None of the cited references teach or even suggest how to provide enhanced enzyme activity, and especially not enhancing enzyme activity a surprising 200-900%.

The 26 April 2007 Rule 132 Declaration showed that the % Brix/mg of alpha-amylase enzyme was higher for enzyme processed according to the present invention relative to the % Brix/mg of unprocessed alpha-amylase enzyme. Since % Brix is a known and accepted measurement of sugar in a solution, and the alpha-amylase enzyme produces sugar in its reaction with starch, a measurement of % Brix/mg of



enzyme is a reasonable measurement of activity. While enzyme activity is often referred to in terms of the amount of enzyme required to produce a certain amount of product, enzyme activity can also be referred to in terms of the amount of product produced per amount of enzyme. In our case, since we are concerned with the activity of the processed enzyme *relative* to the raw enzyme, the '/mg' denominator is not needed. Using this relative activity measurement, the measure of sugar need not be restricted to Brix. Appellant is not bound to use any specific tests and are free to compare the activity of alpha-amylase enzyme, processed according to the method in the present invention, relative to the activity of unprocessed enzyme using any acceptable measurement of product (in this case sugars) in a solution. The 13 March 2008 Rule 132 Declaration confirms this increase in relative activity using % weight, as measured on an HPLC system. Perhaps a better definition of activity is **relative activity**, i.e. the amount of product that results from contacting a certain volume of processed enzyme with substrate at 80 degrees C over 20 minutes relative to the amount of product that results from contacting the same volume of unprocessed enzyme with the same substrate at 80 degrees C over 20 minutes.

Using relative activity as the definition of activity then makes it easy to conclude that alpha-amylase processed according to the current invention is 900% more active relative to unprocessed alpha-amylase (as measured by the amount of product produced per mg of each alpha-amylase). In the 13 March 2008 Rule 132 Declaration, the amount of product (in this case dextrose) produced per milligram of alpha-amylase, processed according to the present invention, is 14 times greater relative to the amount of product produced per milligram of unprocessed alpha-amylase.

The Examiner also argues on page 12 of the Final Office Action that:

Secondly, applicant argues that Lausten's broth cannot be processed through activated carbon by the claimed method due to the presence of cells. Laustsen clearly teaches a filtration step to remove cells. Further it is well known in the art that cells and debris clog filters/membranes and interfere with enzyme activity. Therefore such removal of cells is well within the purview of one of ordinary skill in the art when processing a fermentation broth to obtain a desired protein, i.e. enzyme. Applicant states that Laustsens broth cannot be processed according

to the applicants invention, but it does not appear that applicant actually carried out the claimed method using Laustsen's broth. Applicant did not remove cells from the broth when processing according to their claimed invention. Removing cells when filtering is a routine step well known to those of ordinary skill in the art. Further, for the record, applicant's examples only mix an enzyme solution with activated carbon.

Appellant submits that Laustsen's example 2 does not teach filtration of cells prior to contact with activated carbon. Laustsen's example 2 reads as follows:

A volume of 150 kg Termamyl broth, fermented as described in GB 1,296,839, was diluted to 310 liter with water and 0.300 kg of carbon Picatif FGV 120 together with 6.9 kg of a 45% (w/w) solution of Na.sub.2 Al.sub.2 O.sub.4 from Nordisk Aluminat. pH was adjusted to 10.6, and the microfiltration was done in a continuous mode at 45.degree. C. and 60.degree. C. The solutions were microfiltered on a 1 m2 PallSep PS 10 VMF module (0.45 .mu.m PTFE) and at a TMP (transmembrane pressure) equal or below 0.4 Bar. Average permeabilities were calculated as explained below:

To obtain a better comparison between the continuous filtration experiments by eliminating any minor differences in TMP, the average permeability has been calculated as follows:  $\text{Permeability} = \text{Flux} / \text{TMP}$  ( $\text{Flux} = \text{permeability} \times \text{TMP}$ ). The permeability is a measurement of the amount of fouling, e.g. the higher the permeability the smaller the amount of fouling the better pre-treatment method or filtration process.

Process Permeability Trial temperature L/(m.sup.2 \* hr \* bar) 1 45.degree. C. 138 2 60.degree. C. 157

None of the examples in Laustsen teach a filtration step to remove cells prior to contact with activated carbon. The results in the 13 March 2008 Rule 132 Declaration describe Appellant's observations when replicating Example 2, without applying pressure according to Laustsen, since the claimed invention does not require pressure. Without applying pressure, Appellant could not get the results obtained by Laustsen. This leads one to believe that one skilled in the art would not anticipate that they could follow Example 2 and get an increase in microfiltration process capacity, as is the aim of Laustsen's invention. Nor could one skilled in the art anticipate an increase in specific activity by following this example. Laustsen does not teach or even suggest how to

obtain enhanced enzyme activity.

The examples in the originally filed application used an enzyme solution that was pre-filtered and, thus, no cells were present. Note that the claimed method recites removing cells, if present.

The Examiner states on page 13 of the Final Office Action that the "applicant's distinct objectives, operating conditions, dilution levels and carbon loading are all quite interesting, pertinent and unexpected," however, the examiner goes on to state that claim 31 does not possess such distinct and necessary limitations.

Claim 31 recites the following limitations:

- (a) diluting an enzyme solution comprising glucoamylase with an aqueous solution by a factor of at least three to provide a diluted enzyme solution;
- (b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;
- (c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and
- (d) removing the activated carbon to provide an enzyme solution of enhanced activity.

The experimental evidence of record in the originally filed application and Rule 132 Declarations is commensurate in scope with claim 31, since these limitations were followed. The dilution and carbon loading requirements are recited in claim 31.

On pages 13-14 of the Final Office Action the Examiner indicates that Appellant has failed to show evidence commensurate in scope with the present application.

Applicant's 132 Declaration filed 5/2/2007 fails to show evidence commensurate in scope with the present application. Specifically, applicant has done a comparison, in Fig. 1 and 2 of a native enzyme solution, the enzyme solution processed through activated carbon and that of amylase produced by the method claimed, i.e. dilution and processing through an activated carbon column. It is unclear how one could conclude that such a graph and comparison would

provide unexpected results commensurate in scope with applicant's invention. Applicant also does not provide how such activity is being measured, i.e. specific activity measured in the units as known in Enzymology. Applicant should do a side-by-side comparison of the diluted enzyme without being processed with activated carbon. There is something to be said of an enzyme solution which has been diluted and it's endogenous inhibitors affecting an increase after dilution, i.e. an inhibitor which possesses a low affinity for the enzyme. Changes in activity or inhibition with dilution are a function of the specific enzyme and amount of enzyme in the initial enzyme solution. See Schuster. Further, activated carbon is a known absorbent, therefore the effect may be explained by the fact that the small molecules present in solution are inhibitors of the enzymes therefore binding to the activated carbon, allowing a more pure enzyme to remain. The examiner's position is that a more effective comparison may include additionally the same diluted solution not processed with activated carbon compared to the same volume, amount of enzyme diluted solution which has been processed with activated carbon. It is unclear from the Figure legends in applicants declaration which boxes correspond to the white and grey shaded bars.

In the 13 March 2008 Rule 132 Declaration, on page 13, an experiment is described in which Appellant conducts what the Examiner suggested in a prior Office Action. The chart on page 14 shows unequivocally that the diluted glucoamylase enzyme does not break down the substrate maltodextrin into the products, glucose, as well as the same volume of diluted solution that has been processed with activated carbon according to the present invention.

The 13 March 2008 Rule 132 Declaration shows additional examples to satisfy the Examiner's previous requests. Example 19, on pages 14 – 18 show similar comparisons for fermentation studies. Specifically, Figure 6 shows a comparison of fermentation products for various enzyme formulations. Of note is the glucose production (white bar with horizontal black lines) in a sample of Glucoamylase that has been diluted (1 part glucoamylase and 4 parts buffer), labeled "1:5 Dil GZyme" and processed enzyme (1 part glucoamylase and 4 parts buffer, followed by processing through activated carbon), labeled "Processed Enzyme". Clearly the processed enzyme produces more glucose, which is the product of the enzymatic attack on maltodextrin, than the diluted, unprocessed enzyme.

On page 14 of the Final Office Action, the Examiner states:

In response to applicants arguments regarding "intrinsic activity versus relative activity," relative does not appear to be the same as "intrinsic". An intrinsic activity is something which is inherent to the enzyme.

Examples 1, 2 and 3 show that the intrinsic activity of the amylase enzyme is increased upon subjecting the enzyme to the present invention. In the case of Example 1, raw enzyme contained 2035 U/mL of enzyme activity whereas the enzyme subjected to the present invention contained 11000 U/mL of enzyme activity. Both the raw enzyme and the enzyme treated according to the method of the present invention act on starch at the activities outlined above. Therefore, relative to the enzyme from which the treated enzyme was produced, activity has increased. In addition, the amount of starch hydrolyzed as a function of the amount of raw enzyme in solution, the intrinsic activity, has also increased.

On page 14 of the Final Office Action, the Examiner argues that Laustsen discloses an enzyme dilution of "at least a two part dilution, see example 2, which teaches at least 2 parts." This simply is not an accurate reading of Laustsen.

Laustsen's example 2 reads as follows:

A volume of 150 kg Termamyl broth, fermented as described in GB 1,296,839, was diluted to 310 liter with water and 0.300 kg of carbon Picatif FGV 120 together with 6.9 kg of a 45% (w/w) solution of Na.sub.2 Al.sub.2 O.sub.4 from Nordisk Aluminat. pH was adjusted to 10.6, and the microfiltration was done in a continuous mode at 45.degree. C. and 60.degree. C. The solutions were microfiltered on a 1 m2 PallSep PS 10 VMF module (0.45 .mu.m PTFE) and at a TMP (transmembrane pressure) equal or below 0.4 Bar. Average permeabilities were calculated as explained below:

To obtain a better comparison between the continuous filtration experiments by eliminating any minor differences in TMP, the average permeability has been calculated as follows:  $\text{Permeability} = \text{Flux} / \text{TMP}$  ( $\text{Flux} = \text{permeability} \times \text{TMP}$ ). The permeability is a measurement of the amount of fouling, e.g. the higher the permeability the smaller the amount of fouling the better pre-treatment method or filtration process.

Process Permeability Trial temperature L/(m.sup.2 \* hr \* bar) 1 45.degree.  
C. 138 2 60.degree. C. 157

In the first line, Laustsen specifies that 150 kg Termamyl broth was diluted **to** 310 liters with water. Diluting 150L **to** 310 L requires that one add 160 L of water. This is a dilution of 1:1.07, not 1:2 as stated by the Examiner.

The Examiner also states on page 14 of the Final Office Action:

Applicant also does not show in the submitted Declaration that a dilution of 2:1 is better than or has an unexpected increase of activity compared to a 1:1 dilution.

Appellant has demonstrated this. The Declaration compared the claimed invention to Example 2 of Laustsen, which is a 1:1 dilution using Laustsen's method. Appellant is not required to compare his invention to itself at a 1:1 dilution.

The Examiner further indicates, on page 14 of the Final Office Action, that "Applicant does not specifically claim whether or not the cells are present and/or if they have been filtered out. Thus, applicants arguments are not commensurate in scope with the claimed invention." Claim 31 specifically states "if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells". Therefore, Appellant's arguments are commensurate in scope with the claimed invention.

The Examiner argues on pages 14-15 of the Final Office Action that:

Applicant argues that the Declaration submitted on 5/2/2007 teaches that filtering an enzyme solution through an activated carbon column does not inherently increase enzyme activity. However, as stated above, the showing in the declaration does not accurately show or compare 'dilution, removal of cells if present, and then contacting with the activated carbon' which results in a surprising enhancement of activity. Applicant merely compares the native enzyme solution, the solution with activated carbon and that which has been diluted. Therefore the arguments are not persuasive.

In the 13 March, 2008 Rule 132 Declaration, Appellant compared the claimed invention, a 10 fold (1,000%) diluted enzyme solution (which is free of cells) processed with an amount of activated carbon to provide enhanced activity, to the cited prior art teaching (1) undiluted enzyme and (2) undiluted enzyme solution merely contacted with

activated carbon. Furthermore, in the same Rule 132 Declaration, Appellant has compared the claimed method (dilution of purified enzyme processed with amount of activated carbon to effect enhanced enzyme activity) with a merely diluted enzyme, which conclusively confirms that mere dilution does not result in enhanced enzyme activity. The 13 March, 2008 Rule 132 Declaration demonstrates that the claimed invention provided a surprisingly far greater enzyme activity compared to the enzyme activity of the merely diluted enzyme. Appellant requests, again, that the Board consider the experimental evidence of record and withdraw the prior art rejections of record.

On page 15 of the Final Office Action, the Examiner argues that:

It is noted that applicant has submitted many documents teaching away from applicants claimed invention, i.e. attempting to overcome the inherency rejection and to show unexpected results.

Regarding applicant's arguments directed towards the Shenoy reference, i.e. that Shenoy does not teach purification resulting in activity three times higher than the original non-purified glucoamylase, rather compared to a parent strain. While this has been considered, applicant does not specifically claim, nor show in the Declaration, purification of an original non-purified enzyme. Applicant actually compares in the declaration dated 5/2/2007, an already purified commercial enzyme to a diluted enzyme purified by the claimed method. Thus, applicant's arguments are not commensurate in scope with the present invention.

As stated in Appellant's response of March 2008, the Appellant's invention requires purification, i.e. removal of cells, only if cells are present before application of the activated carbon. Thus, if an already purified enzyme solution is utilized in the claimed invention, then the step of removing cells is not required since they are already removed. In either case, a purified enzyme solution free of cells is enhanced according to the claimed steps. An already purified enzyme can absolutely be used to practice the claimed invention. None of the cited references teach or even suggest taking a commercially available purified enzyme and subjecting it to the claimed 3-fold dilution and activated carbon steps to enhance the enzyme activity.

The novelty of Appellants' invention is that the enzyme activity can be greatly enhanced, surprisingly greater than 200%, by the claimed steps of diluting an enzyme

solution (which has been purified by removing cells) with an aqueous solution by at least 3 times, and then processing with an amount of activated carbon and for a time sufficient to effect an enhancement of enzyme activity. As correctly pointed out by the Examiner, the examples used purified enzyme, i.e. no cells present. The step of removing cells if present does not alter this experimental evidence, since the cells are absent either way. The enzyme activity of the purified enzyme was conducted according to the claimed steps and, thus, the experimental evidence is commensurate in scope with the claimed invention and must be considered.

The cited prior art does not teach or suggest the claimed steps. Furthermore, the prior art does not teach or even suggest the surprising 200% to 900% increase in enzyme activity.

Appellant respectfully submits that the Examiner improperly cites the teachings of Shenoy by assuming that the 3-fold increase in activity is due to purification. Shenoy does not explicitly state that the enhancement of activity is due to purification. Based on the general knowledge in the art, as represented by the numerous references now of record, purification mostly results in reduced enzyme activity and at best the same enzyme activity. Thus there is no support in art or Shenoy for the Examiner's position that Shenoy teaches enhanced activity can be obtained by purification. While the wording in Shenoy is vague, one of ordinary skill in the art would properly interpret the meaning of Shenoy's statements that the enzyme activity enhancement is due to genetic modification in the organism rather than due to purification.

In the second part of the Examiner's argument, reference is made to the May 2, 2007 Rule 132 Declaration, wherein "purification" of an already purified enzyme is discussed. To be clear, the Declaration shows activities of the enzymes before and after they have been processed using the presently claimed technology. The activity of the untreated enzyme, which is a commercially available purified enzyme, has been compared to the activity of this same enzyme after it has been subjected to the claimed process. Since the commercial enzyme has been purified before being subjected to the claimed process, one of ordinary skill in the art would conclude that further purification



would have little effect on the enzyme. However, this is not the case. Evidence in the May 2, 2007 Rule 132 Declaration shows that decreasing the enzyme concentration to  $1/10^{\text{th}}$  the concentration of the original commercial enzyme and subjecting the enzyme to the presently claimed process increases the amount of product relative to the mass of enzyme used. That Appellant can accomplish this dramatic increase in enzyme activity on an already purified enzyme further supports the claimed invention. Simple removal of inhibitors and other "non-essential" proteins would have already been largely accomplished during the manufacturer's purification step, which rules out the Examiner's other hypothesis that enhancement is due to removal of these inhibitors. To see a further 10-fold improvement in activity after Appellant has processed and already purified enzyme is therefore unexpected and fully rebuts the prior art rejections of record.

On page 16 of the Final Office Action, the Examiner argues that:

Applicant argues that the art does not teach or suggest the claimed process of contacting a diluted enzyme solution to activated carbon, in which the cells have been removed. Laustsen by themselves do teach such a process.

The present invention claims the specific conditions required to create the desired effect of the invention. Laustsen does not claim dilution by a factor of at least three, nor does Laustsen claim treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect an enhancement in enzyme activity. This is because neither Laustsen, nor anyone else of ordinary skill in the art could conceive that by following the steps outlined in the present invention an increase in activity of more than 200% would be observed.

If Laustsen did teach the claimed method, Laustsen surely would have disclosed those method steps and claimed them because the claimed invention surprisingly results in a large reduction in the amount of required enzymes and corresponding reduction in costs associated with purchasing and producing enzymes for use in, but not

limited to, renewable fuels production.

In view of many difference between the claimed invention and the combination of Lausten and Schuster, and the unexpected advantages of the claimed invention, the methods recited in claims 2-7, 31-33, 39, 40, 46-52, 63, 64 and 66 are patentable over the combination of Lausten and Schuster.

**The product of claims 42-44 is also patentable over the combination of Lausten and Schuster.**

The product recited in claims 42-44 has enhanced enzyme activity. Lausten and Schuster do not teach or suggest how to make an enzyme solution having enhanced activity. Lausten and Schuster merely teach purified enzyme solutions, which do not have enhanced enzyme activity, as discussed above. Furthermore, the unexpected advantages of the claimed product are discussed above in regards to the Rule 132 Declarations. In view of the differences between the claimed invention and the combination of Lausten and Schuster, and the unexpected advantages of the claimed invention, the products recited in claims 42-44 are patentable over the combination of Lausten and Schuster.

- D. **Claims 2-7, 12, 13, 31-33, 39, 40, 42-44, 46-61, 63, 64 and 66 are patentable under 35 U.S.C. § 103(a) over Lausten in view of Schuster, Shenoy and [http://22.ap-lab.com/circular\\_dichroisms.htm](http://22.ap-lab.com/circular_dichroisms.htm).**

Claims 12, 13, 54-58 recite **methods**, which stand or fall together for purposes of this appeal.

Claims 2-7, 31-33, 39, 40, 46-53, 59-61, 63, 64 and 66 recite **methods**, which stand or fall together for purposes of this appeal.

Claims 42-44 recite **products**, which stand or fall together for purposes of this appeal.

Claims 2-7, 12, 13, 31-33, 39, 40, 42-44, 46-61, 63, 64 and 66 are patentable

under 35 U.S.C. § 103(a) over Lausten in view of Schuster, Shenoy and [http://22.ap-lab.com/circular\\_dichroisms.htm](http://22.ap-lab.com/circular_dichroisms.htm).

**The methods of claims 2-7, 31-33, 39, 40, 46-53, 59-61, 63, 64 and 66 are patentable over the combination of Lausten, Schuster and Shenoy.**

Claims 2-7, 31-33, 39, 40, 46-53, 59-61, 63, 64 and 66 recite methods, which are not taught or suggested by the combination of Lausten and Schuster for the many reasons provided above, including the detailed discussion of the Rule 132 Declarations of record. Shenoy does not provide the deficiencies of Lausten and Schuster for the following reasons.

Shenoy discusses the purification and properties of glucoamylases from different fungal sources, using circular dichroism (CD) to examine effects of pH, T, substrate and denaturants. The binding kinetics and reaction mechanism are also discussed.

Shenoy notes that near-UV CD at optimal pH depends on the fungal source. *A. niger* and *A. candidus* GA showed extra peaks at 272-275 nm; troughs observed with *A. niger* and *A. candidus* were not observed with *Rhizopus* species. Glucoamylases from different sources had different aromatic amino acids and cysteine content. The bottom line is that the species used to produce the glucoamylase affects the near-UV CD spectrum. The far-UV spectra also depend on species. Slight differences in magnitude and band position were observed in the 208-210nm and 217-220nm range. Some of the subtle shifts in band position are thought to be due to carbohydrate moieties in the enzyme. Enzymes from different species contain the same amount of alpha helix, but differ in beta-sheet structure. Shenoy teaches that pH affects secondary structure and activity of glucoamylase. Maximum activity occurred at pH 4.8, which corresponded to the highest percentage of alpha-helix and lowest percentage of beta-sheet in all three species - *A. niger*, *A. candidus*, and *Rhizopus*. The lowest activity (pH 10)

corresponded to higher levels of either beta-sheet or gamma (disordered structure) content. A higher pH causes ionization of tyrosine, leading to unfolding and loss of activity, consistent with involvement of ionic linkages in the native conformation. Shenoy also shows that increased temperature reduces helical content, ultimately leading to unfolding at higher temperatures (> 60°C). Exposure to urea reduced alpha-helix content; this paralleled a decrease in activity. Addition of substrate also reduced the alpha-helix content, while increasing the beta-sheet content. However, near-UV bands were unaffected. Carbohydrate groups were necessary for stability, but not for activity. Perhaps the CHO moieties stabilize the enzyme against heat denaturation. Reduction of the enzyme (with and without periodate treatment) confirmed importance of the CHO for structural stability.

Essentially, Shenoy has used CD to show structural differences between different sources of glucoamylase, and structural changes that occur as activity changes. In other words, following a change in activity due to a change in pH, temperature, or chemical treatment, CD can be used to elucidate the structural changes that result from such a treatment or process. One cannot, however, use a change in CD spectrum to develop a process, or to predict that a particular treatment would lead to a specific change in activity. Indeed, as noted by Alliance Protein Laboratories on their website, even though one can use CD to establish the approximate percentage of alpha-helix content, "it cannot determine which specific residues are involved in the alpha-helical portion". Appellant has also shown that the alpha-helix content of different amylases is highly dependent upon the source enzyme (Figure 3 of instant specification), but all of these enzymes are highly active, so the CD spectrum cannot be a unique predictor of activity. Thus, even though the secondary and tertiary structures of these enzymes are different, they are all active. Therefore, a specific secondary structure or a specific tertiary structure is not essential for activity, but a range of such structures may produce an "active" conformation. Shenoy simply does not teach any methods for increasing enzyme activity.

The Examiner states that the "information found at [http://22.ap-lab.com/circular\\_dichroisms.htm](http://22.ap-lab.com/circular_dichroisms.htm) teaches that any change in spectral range appears to be an inherent property of purification, i.e. structural change, of a protein." However, this reference, and Shenoy, do not teach that a change in spectral range results in enhanced enzyme activity. Indeed, this reference actually teaches that "Often it is necessary to demonstrate that different lots of a protein have equivalent conformations, for example after a scale-up in the purification process or to qualify a new manufacturing site, and CD can be a good tool for this." In this same section, the reference goes on to teach that "far-UV spectra show that the recombinant form of an enzyme clearly does not have the same secondary structure as the natural protein (i.e. the recombinant protein is not properly folded)." Thus, this reference clearly teaches that a change in CD is to be avoided, which is in a direction away from the claimed invention.

Appellant's arguments are not confusing to one of ordinary skill in the art. As accurately stated, the goal of purification is to avoid a structural and spectral change. This is a well known fact, which is supported by the numerous references made of record in Appellant's 2 May 2007 Response and discussed below.

Appellant's invention is not mere purification. The claimed invention recites steps that are not described in the prior art and are not mere purification. As stated previously and confirmed by the experimental evidence of record, the claimed invention results in a dramatic and unexpected increase in enzyme activity. As a proposed explanation, without being bound by any theory, Appellant also submitted the structural and spectral data.

In a typical purification process, as understood by those of ordinary skill in the art, the goal is to avoid a change in structure, so that activity is mostly preserved. The claimed process is not typical, and in fact, might not even be classified as purification, because an embodiment of the claimed process appears to cause a change in structure, and the resulting structure has higher activity than the original (unprocessed) enzyme. This is why Appellant refers to catalytic modification of the protein, without

being bound by any theory. Thus, Appellant is reciting in certain claims that the change in structure, as evidenced by the change in CD/UV spectrum, is evidence of the efficacy of the claimed process, because this structural change is associated with an increase in activity, unlike in a conventional purification, where a change in structure leads to a decrease in activity.

Appellant respectfully submits that this statement is incorrect. The Examiner contends that treatment of enzymes with activated carbon, or indeed, any purification step, should automatically lead to an enhancement of activity. However, Lausten is silent on this matter, and previously Bailey (U.S. Patent No. 4,204,041 cited in 10 January 2007 Office Action) recorded a decrease in activity after enzymes were "mixed" with activated carbon. It is generally known throughout the art that purification leads to a decrease in enzyme activity. Enhancement of enzyme activity is not an inevitable outcome of purification as is apparently believed by the Examiner. To support Appellant's position, the following evidence was presented.

Every basic course in biochemistry and proteomics teaches protein purification, and the basic tenet is that purification is a challenge, fraught with many adverse outcomes. While it may be possible to overcome some of these challenges, some proteins cannot be effectively isolated in an active form.

The Cornell lab manual for BIOBM330 discusses strategies for protein purification. (<http://instruct1.cit.cornell.edu/Courses/biobm330/protlab/Strategy.html>) The manual specifically states:

Because of structural and functional differences between proteins, an ideal sequence of steps for one protein will, quite possibly, be unsuccessful for another. A knowledge of the theoretical basis of each procedure will allow the researcher to choose an initial sequence of techniques with which to attempt any given purification. However, the development of an optimised protocol involves considerable trial-and-error experimentation to assess the potential of each step

The manual also specifically states:

Note that the yield of enzyme after a particular purification procedure may be low not because the procedure is failing to purify that protein, but because it is causing some inactivation of the enzyme.

Based on these statements, it is apparent that the procedure is not automatic – the fact that a procedure works provides no guarantee that it will work for another enzyme.

Furthermore, even maintenance of activity is difficult, and enhancement of activity is not automatic.

Instructional materials for the MATC Biotechnology program in Madison, WI discuss methods and goals for protein purification.

[http://matcmadison.edu/biotech/resources/proteins/labManual/chapter\\_1.htm](http://matcmadison.edu/biotech/resources/proteins/labManual/chapter_1.htm)

They state:

The ideal purification strategy has the following goals: maximum recovery of the target protein; minimal loss of biological activity; and maximum removal of contaminating proteins, as well as low cost...

Note that one of the goals is to minimize loss of activity – enhancement of activity is difficult, although specific activity may increase, depending upon one's ability to concentrate the desired protein.

There is significant supporting evidence in the research literature as well, in spite of the fact that the tendency would be to publish successful separation methods, and not report the failures. For example, Wingfield et al. (Eur. J Biochem. 180.23-32 (1989) studied the purification of a wild-type and recombinant aminopeptidase, and noted significant loss of activity during purification, likely due to loss of essential metal ions. On page 28, they state: "The enzyme, fully active before the separation step, was completely inactive after the separation..."

Mackay et al. (Fundamental and Applied Toxicology, vol 30, pp23 – 30, 1996) studied a protein with neuropathy target esterase activity, and noted (page 26):

This suggests there was an appreciable deactivation of the enzyme during purification. This trend was seen throughout the purification process; large

amounts of protein without NTE activity were removed from the active fraction with no appreciable increase in NTE specific activity.

These examples, among many others, demonstrate that the purification/separation method does not automatically lead to an enhancement of activity. Indeed, these authors report loss of activity during purification, which is indeed a common problem, and a challenge to be overcome, as noted in the instructional materials at Cornell and MATC. Thus, the examiner's claim that enhancement of enzyme activity is an inherent outcome of purification is wholly inconsistent with the science of protein purification.

The Examiner contends that processing with activated carbon, as a well-established purifying agent, should automatically enhance enzyme activity. Again, the literature demonstrates that such an outcome is not automatic. For example, Pimenov et al. (Sep. Sci. Technol., 36(15), 3385-3394, 2001) studied microbial growth and survival following contact with activated carbon. The authors discovered that contact with activated carbon had a significant adverse impact on microbial growth and enzyme activity:

"The experiments provided conclusive evidence that adhesion of microbes onto the activated carbon fiber directly influences bacterial metabolism". In this case, "The esterase metabolic activities of the adsorbed microorganisms were measured and were found to diminish rapidly and cease completely in less than 24 hours."

In an undated report, Hydamaka et al. from the Food Science Department at the University of Manitoba evaluated the use of activated carbon to control color problems and enzymatic browning in recycled food process waters. The observed that with a proper loading of activated carbon, the activity of the phenolase enzyme responsible for browning could be reduced to zero.

In conclusion, Appellant contends that the above evidence and references clearly demonstrate that processing with activated carbon alone does not lead to an enhancement in enzyme activity.



**Appellant Addresses Below Each Argument  
Raised by the Examiner in the Final Office Action.**

On pages 16-17 of the Final Office Action the Examiner argues that:

Applicant argues that one of skill in the art would not have been motivated to dilute an enzyme prior to purification with activated carbon because the art does not suggest so. However, Laustsen does teach dilution prior to purification. In response to applicant's arguments, that there is no motivation or teaching/suggestion, applicant is advised that KSR forecloses the argument that a specific teaching, suggestion or motivation is required to support a finding of obviousness. See the recent board decision *Ex parte Smith*, --USPQ2d--, slip op at 20, (Bd. Pat.App Y Interf. June 25, 2007)(citing KSR, 82 USPQ2d at 1396) (available at <http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf>)

Appellant did not only argue "that one of skill in the art would not have been motivated to dilute an enzyme prior to purification with activated carbon because the art does not suggest so" as alleged by the Examiner. Appellant also pointed out how mere dilution of Laustsen's method still does **not** result in the claimed invention. The Rule 132 Declaration submitted on 17 March, 2008 comparing the claimed invention to the specific teachings in Laustsen confirms such.

Furthermore, this Rule 132 Declaration and the previous Rule 132 Declaration of record confirm the unexpected advantages of the claimed invention compared to the prior art. Appellant is not required to compare his invention to a theoretical method that does not exist. Appellant is only required to compare his invention to what is known in the art, i.e. actual teachings, such as an experimental example. KSR specifically states that unexpected advantages overcome a prima facie case of obviousness. Thus, according to the holding in KSR, the Section 103 rejections should be withdrawn in view of the unexpected results confirmed by the experimental evidence of record in the originally filed application and Rule 132 Declarations of record.

In view the many differences between the claimed invention and the combination of Lausten, Schuster and Shenoy, and the unexpected advantages of the claimed

invention, claims 2-7, 31-33, 39, 40, 46-53, 59-61, 63, 64 and 66 recite methods that are patentable over the combination of Lausten, Schuster and Shenoy.

**The methods of claims 12, 13, and 54-58 are patentable over the combination of Lausten, Schuster and Shenoy.**

The methods of claims 12, 13 and 54-58 are not taught or suggested by the combination of Lausten, Schuster and Shenoy for the reasons provided above, and for the following additional reasons. Appellant responds directly to the Examiner's arguments in Final Office Action as follows.

The Examiner, on pages 15-16 of the Final Office Action, argues that Appellant's arguments directed to spectral change continue to be confusing:

Applicant's arguments directed to spectral change continue to be confusing. Applicant is arguing that it is the goal of purification to avoid a structural and spectral change, yet claim a spectral change, which is distinct from the "raw enzyme solution", as in claim 11,12,13,30. The argument is contradictory. The examiner is well aware of the fact that CD is used after the fact to determine alteration in the structure. Applicant argues that their method is unique in that it leads to a change in protein structure. Applicant argues that their method is unique in that it leads to a change in protein structure, due to catalytic modification of the protein. Shenoy also teach that the catalytic activity of a protein, i.e. enzyme is related to its "active" conformation, i.e. secondary and tertiary structure. They state that the ideal purification would preserve protein structure and avoid changes in spectral properties, yet claim that their method is unique in that it leads to a change in protein structure, due to catalytic modification of the protein. This argument is not understood.

It is true that the goal of purification is to avoid a structural and spectral change, as supported by the many references now of record and discussed fully in Appellant's 2 May 2007 Response.

The Examiner's arguments would be well founded if the Appellant's invention were solely purification. Appellant is arguing that the present invention is not solely purification. Purification alone could not explain the dramatic and unexpected increase

in enzymatic activity. Therefore, since the present invention does not result solely in purification, there are some other phenomena occurring. These phenomena can, in part, be explained by a shift in CD and UV spectra.

As stated in Appellant's 17 March 2008 response, in a typical purification process, as understood by those of ordinary skill in the art, the goal is to avoid a change in structure, so that activity is mostly preserved. In most cases, a change in structure will decrease, if not completely destroy, activity. In very few cases can a change in structure increase activity. The claimed process is obviously not a typical change in structure, nor is it a typical purification, because an embodiment of the claimed process appears to create a structure that has a higher activity than the original (unprocessed) enzyme. This is why Appellant refers to catalytic *modification* of the protein, without being bound by any theory. Thus, Appellant is reciting in certain claims that the change in structure, as evidenced by the change in CD/UV spectrum, is evidence of the efficacy of the claimed process, because this structural change is associated with an increase in activity, unlike in a conventional purification, where a change in structure leads to a decrease in activity.

On page 16 of the Final Office Action, the Examiner argues that:

In response to applicants previous argument dated 10/19/2006 p. 11 and 7/21/2009 of the response, applicant had argued that they do not claim a specific CD spectra, but rather that there had been a change in structure as supported by CD spectra. The examiner's argument, "Applicant absolutely claims a specific CD spectra in claims 12-15, thus applicants arguments are not supported by the claims". Applicant now argues in the response filed 5/2/2007 that this statement is not understood and that claims 11-15 show changes in the defined spectra in the claims. Therefore, in claims 12, applicant does claim a specific CD spectral range.

This statement is not understood by Appellant. Reference to claims 11 to 14 and 54 to 58 listed below recite **changes in the defined spectra** as highlighted by Appellant's underlining in the claims. In other words, the spectra for the enzyme solution produced according to the claimed method is different from the conventional

enzyme solution in the recited spectral range, which is very different from claiming a specific spectral range.

11. A method as defined in claim 31 wherein said enzyme solution of enhanced activity has a spectrum selected from Far UV (CD) and UV visible spectra distinct from said raw enzyme solution.
12. A method as defined in claim 11 wherein said enzyme solution of enhanced activity shows a relative absorbance intensity lower than said raw enzyme solution, in the CD spectral range of 205-230 nm.
13. A method as defined in claim 11 wherein said enzyme is alpha-amylase and said enzyme solution of enhanced activity has a Far UV (CD) spectrum minimum ellipticity shifted by at least 1 nm, from the raw enzyme solution, in the range between 205-230 nm.
14. A method as defined in claim 31 wherein said enzyme solution of enhanced activity has a UV-visible spectrum maximum peak at least 30 nm lower than said raw enzyme solution.
54. A method as defined in claim 46 wherein said enzyme solution of enhanced activity has a spectrum selected from Far UV (CD) and UV visible spectra distinct from said raw enzyme solution.
55. A method as defined in claim 54 wherein said enzyme solution of enhanced activity shows a relative absorbance intensity lower than said raw enzyme solution, in the CD spectral range of 205-230nm.
56. A method as defined in claim 54 wherein said enzyme is alpha-amylase and said enzyme solution of enhanced activity has a Far UV (CD) spectrum minimum ellipticity shifted by at least 1nm, from the raw enzyme solution, in the range between 205-230 nm.
57. A method as defined in claim 46 wherein said enzyme solution of enhanced activity has a UV-visible spectrum maximum peak at least 30 nm lower than said raw enzyme solution.
58. A method as defined in claim 46 wherein said enzyme is alpha-amylase and said enzyme solution of enhanced activity has a maximum spectral absorption peak over the range 340 to 360 nm.

It is clear that these claims define a change in spectral properties, not an absolute spectrum as stated by the Examiner.

Respectfully, if Appellant claimed a specific CD spectra the claim would look something like this:

A method as defined in claim 31 wherein said enzyme solution of enhanced activity shows an ellipticity per residue of between  $-35 \times 10^{-3}$  and  $-45 \times 10^{-3}$  at 206 nm

Or

A method as defined in claim 31 wherein said enzyme solution of enhanced activity shows a Molar Ellipticity of -110 at 300nm

Also, one of ordinary skill in the art would not claim a "spectral range" since the "spectral range" is the independent variable. The spectral range is the range of wavelengths of light at which ellipticity is measured, therefore claims would likely be made on either a combination of dependent and independent variables or on the dependent variable which is molar ellipticity or ellipticity per residue rather than only the spectral range itself.

Reading claims 11-14 and 54-58 therefore indicates to one skilled in the art that the enzyme that has been subjected to the present invention has "visible spectra distinct from said raw enzyme solution" and "relative absorbance intensity lower than said raw enzyme solution in the spectral range of 205 – 230 nm". Appellant is not claiming a spectral range of 205 – 230 nm. Appellant is simply claiming that in the 205 – 230 nm range, the molar ellipticity or relative absorbance intensity of the treated enzyme is lower than the molar ellipticity or relative absorbance intensity of the raw enzyme in the same 205 – 230 nm spectral range.

It is impossible for Appellant to claim a specific spectra because the spectra of the enzyme subjected to the present invention is dependent on the spectra of the raw enzyme. Appellant is claiming that between 205 and 230 nm, the processed enzyme will have a lower relative absorbance intensity than the raw enzyme.

Each of claims 11-14 and 54-58 indicate changes in spectra relative to the unprocessed raw enzyme.

In view the many differences between the claimed invention and the combination of Lausten, Schuster and Shenoy, and the unexpected advantages of the claimed invention, claims 12, 13, and 54-58 recite methods that are patentable over the combination of Lausten, Schuster and Shenoy.

**The product of claims 42-44 is also patentable over the combination of Lausten, Schuster and Shenoy.**

The product recited in claims 42-44 has enhanced enzyme activity. Lausten, Schuster and Shenoy do not teach or suggest how to make an enzyme solution having enhanced activity. Lausten, Schuster and Shenoy merely teach purified enzyme solutions, which do not have enhanced enzyme activity, as discussed above. Furthermore, the unexpected advantages of the claimed product are discussed above in regards to the Rule 132 Declarations. In view of the differences between the claimed invention and the combination of Lausten, Schuster and Shenoy, and the unexpected advantages of the claimed invention, the products recited in claims 42-44 are patentable over the combination of Lausten, Schuster and Shenoy.

**E. Claims 2-7, 11, 14, 31-33, 39, 40, 42-44, 46-58, 64 and 66 are patentable under 35 U.S.C. § 103(a) over Aikat in view of each of Lausten and Schuster.**

Claims 11, 14, 54-58 recite **methods**, which stand or fall together for purposes of this appeal.

Claims 2-7, 31-33, 39, 40, 42-44, 46-53, 59-61, 63, 64 and 66 recite **methods**, which stand or fall together for purposes of this appeal.

Claims 42-40 recite **products**, which stand or fall together for purposes of this

appeal.

Claims 2-7, 11, 14, 31-33, 39, 40, 42-44, 46-58, 64 and 66 are patentable under 35 U.S.C. § 103(a) over Aikat in view of Lausten and Schuster.

**The methods of claims 2-7, 31-33, 39, 40, 46-53, 59-61, 63, 64 and 66 are patentable over the combination of Aikat, Lausten and Schuster .**

Claims 2-7, 31-33, 39, 40, 46-53, 59-61, 63, 64 and 66 recite methods, which are not taught or suggested by the combination of Lausten and Schuster for the many reasons provided above, including the detailed discussion of the Rule 132 Declarations of record. Aikat does not provide the deficiencies of Lausten and Schuster for the following reasons.

Appellant responds directly to the Examiner's arguments in Final Office Action as follows. The Examiner argues on pages 9-14 of the Final Office Action that:

Applicant claims a method of enhancing the intrinsic activity of an enzyme solution, specifically a hydrolase, by treating with a purifying agent, activated carbon, and further removing the activated carbon from the enzyme solution by centrifugation. The purified enzyme solution is said to have a CD and UV spectrum distinct from that of the raw enzyme solution, specifically 30 nm less and the enzyme to carbon ratio is not to exceed 15:1.

Aikat et al. teach the purification of protease by activated charcoal, i.e. activated carbon. They demonstrate the purification by activated charcoal in terms of fold purification and by electrophoretic analysis (see introduction). The enzyme solution was mixed with activated charcoal and allowed to react for a specific period of time prior to centrifugation, thus removing the activated carbon, at which time the supernatant was examined by spectroscopy. Further analysis was carried out by electrophoresis (see p. 296). The enzyme solution (1 ml) was treated with 50 to 150 mg of activated charcoal, although 75 mg of charcoal was selected as their optimum ratio. By gel analysis they observed the removal

of almost all of the smaller proteins, confirming the purifying action of activated charcoal.

Further, Aikat diluted the crude enzyme solution 10 times to bring its absorbance within the range of that of charcoal-treated enzyme, which shows distinct troughs at 260 nm and a peak at 280nm. In the crude diluted solution there appeared to be a peak at 260 nm and no valley (see p.299 to 300).

Aikat does not teach diluting the raw enzyme solution prior to treating with a purifying agent. However, as stated above Laustsen teach diluting an enzyme solution prior to treating the activated carbon.

Schuster teaches that enhancement of enzyme activity by treating with charcoal (results and discussion section) as well as diluting with water prior to treating with charcoal.

Thus, at the time of the invention it would have been obvious to one of ordinary skill in the art to dilute an enzyme solution prior to treatment because the prior art teaches dilution prior to treatment with activated carbon. Moreover, at the time of the claimed invention, one of ordinary skill in the art would have been motivated to have diluted an enzyme solution prior to purification with activated carbon with a reasonable expectation for successfully enhancing the intrinsic activity of such solution because the art teaches such success when using the claimed enzyme solution and purifying agent.

The experimental evidence demonstrates that merely contacting the enzyme solution with activated carbon does not result in an increase in enzyme activity. See the graphs in the 26 April 2007 Rule 132 Declaration of record, center bars (no dilution, contact with activated carbon), which are the same height as the left most bars representing the original enzyme solution. In contrast, when the enzyme solution is diluted with an aqueous solution, cells are removed when present, and then contacted with the claimed amount of activated carbon, an astonishing 10 fold (1,000%) increase in enzyme activity is obtained. See the graphs in the Rule 132 Declaration, right most bars of each three bar set. If the enzyme activity did not increase 10 fold, the right most bars would be 1/10 the height of the left most bars for each set of three bars. For this reason alone the Section 103 rejection should be withdrawn.

The Examiner has acknowledged that Aikat does not teach diluting the raw enzyme solution prior to treatment with an activated carbon. Appellant respectfully



submits that one of ordinary skill in the art would not have been motivated to dilute an enzyme solution prior to purification with activated carbon with a reasonable expectation for successfully enhancing the intrinsic activity of such solution, because the art does not teach such success when using the claimed enzyme solution or any other enzyme solution and activated carbon.

Furthermore, Laustsen and Schuster do not teach the combination of the claimed steps of diluting to a level of at least 3 times, removing cells if present, contacting with the claimed amount of activated carbon for a time sufficient to form an enzyme solution having enhanced activity, and removing the activated carbon. As discussed previously, Laustsen teaches diluting an enzyme broth containing cells only to a level of about 2.2 times (110%) and contacting the solution containing cells to carbon. None of the cited references, alone or in any combination, teach the claimed steps of dilution at least 3 times (200%), removing cells if present, and then contacting the diluted solution with activated carbon in an amount and for a time to effect enhancement of the enzyme activity. For this reason alone, the Section 103 rejection should be withdrawn.

The cited references do not teach or suggest the unexpected results of the claimed invention. The key to the results of Aikat is their electrophoresis results. The images of the crude protein and the charcoal-treated proteins show that the smaller proteins were almost completely removed by the activated carbon. This is clearly a quintessential case of protein purification, not dilution and enhancement according to the present invention.

By comparison, an embodiment of the claimed process results in an extra band when glucoamylase is treated, and the smaller proteins are more abundant when amylase is treated (shown as A, A', B, B' in Figure 3). The observations are consistent with a structural change in the native protein, but not consistent with the simple purification described by Aikat. It can therefore be concluded that Aikat have performed a simple purification – small proteins, debris, and extracellular nucleic acids have been removed by adsorption and/or size exclusion, while leaving the treated protein unaffected. In contrast, the claimed process leads to transformation of the enzyme and

dilution accompanied by enhanced activity.

There is nothing within Laustsen or Schuster to suggest an increase in activity arising from treatment with activated carbon and the Examiner previously acknowledged that Laustsen does not "specifically state the enhancement of enzyme activity." See page 5 of the 10 January 2007 Office Action.

The Examiner now simply concludes that "at the time of the claimed invention, one of ordinary skill in the art would have been motivated to have diluted an enzyme solution prior to purification with activated carbon with a reasonable expectation for successfully enhancing the intrinsic activity of such solution because the art teaches such success when using the claimed enzyme solution and purifying agent." The art absolutely does **not** teach that the enzyme activity can be enhanced, as admitted previously by the Examiner, and surely not by an astounding 200 to 900%.

Indeed, if the Examiner's conclusion was correct, previously cited Bailey (U.S. Patent No. 4,204,041 cited in 10 January 2007 Office Action) should have seen an increase in activity when they contacted enzyme with activated carbon during immobilization, but they did not – in fact, in their patent, they explicitly state that there was no benefit observed when some types of activated carbon were used in their process. Furthermore, the Rule 132 Declaration dated 26 April 2007 absolutely confirms that merely contacting the enzyme with activated carbon does not result in enhanced enzyme activity.

In view the many differences between the claimed invention and the combination of Aikat, Lausten, and Schuster, and the unexpected advantages of the claimed invention, claims 2-7, 31-33, 39, 40, 46-53, 59-61, 63, 64 and 66 recite methods that are patentable over the combination of Aikat, Lausten, and Schuster.

**The methods of claims 11, 14, and 54-58 are patentable over the combination of Aikat, Lausten, and Schuster.**

The methods of claims 11, 14 and 54-58 are not taught or suggested by the combination of Lausten and Schuster for the reasons provided above. Aikat does not provide the deficiencies of Lausten and Schuster.

None of the cited references disclose a process to produce **changes in the defined spectra** recited in claims 11, 14 and 54-58. The spectra for the enzyme solution formed according to the claimed invention is different from the conventional enzyme solution in the recited spectral range.

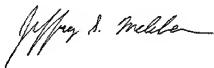
**The product of claims 42-44 is also patentable over the combination of Aikat, Lausten, and Schuster.**

The product recited in claims 42-44 has enhanced enzyme activity. Aikat, Lausten, and Schuster do not teach or suggest how to make an enzyme solution having enhanced activity. Aikat, Lausten, and Schuster merely teach purified enzyme solutions, which do not have enhanced enzyme activity, as discussed above. Furthermore, the unexpected advantages of the claimed product are discussed above in regards to the Rule 132 Declarations. In view of the differences between the claimed invention and the combination of Aikat, Lausten, and Schuster, and the unexpected advantages of the claimed invention, the products recited in claims 42-44 are patentable over the combination of Aikat, Lausten, and Schuster.

Conclusion

In view of the lack of *prima facie* cases of obviousness, the many differences between the claimed invention and the cited references, and the unexpected advantages of the claimed invention, it is believed that this application clearly and patentably distinguishes over the combination of the cited references and is in proper condition for allowance. Accordingly, Appellant respectfully requests that the Board allow claims 2-7, 11-14, 31-36, 39, 40, 42-64, and 66 over the cited reference.

Respectfully submitted,  
Manelli Denison & Selter, PLLC

A handwritten signature in black ink, appearing to read "Jeffrey S. Melcher". The signature is fluid and cursive, with a long horizontal stroke at the end.

By

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(viii) Claims Appendix:

1. (Cancelled)
2. (Previously Presented) A method as defined in claim 31 wherein said aqueous solution comprises an aqueous buffer solution.
3. (Previously Presented) A method as defined in claim 31 wherein said aqueous solution comprises water.
4. (Previously Presented) A method as defined in claim 31 comprising passing said raw enzyme solution through a column containing an effective amount of said activated carbon.
5. (Previously Presented) A method as defined in claim 31 wherein said activated carbon is removed by a method selected from the group consisting of filtration and centrifugation.
6. (Previously Presented) A method as defined in claim 31 wherein said raw enzyme solution is diluted with water to provide a diluted raw enzyme solution.
7. (Previously Presented) A method as defined in claim 31 wherein said raw enzyme solution is diluted with an aqueous buffer solution to provide a buffered diluted raw enzyme solution.
8. (Cancelled)
9. (Canceled)
10. (Cancelled)

11. (Previously Presented) A method as defined in claim 31 wherein said enzyme solution of enhanced activity has a spectrum selected from Far UV (CD) and UV visible spectra distinct from said raw enzyme solution.
12. (Original) A method as defined in claim 11 wherein said enzyme solution of enhanced activity shows a relative absorbance intensity lower than said raw enzyme solution, in the CD spectral range of 205-230nm.
13. (Original) A method as defined in claim 11 wherein said enzyme is alpha-amylase and said enzyme solution of enhanced activity has a Far UV (CD) spectrum minimum ellipticity shifted by at least 1nm, from the raw enzyme solution, in the range between 205-230 nm.
14. (Previously Presented) A method as defined in claim 31 wherein said enzyme solution of enhanced activity has a UV-visible spectrum maximum peak at least 30 nm lower than said raw enzyme solution.

Claims 15-30 (Canceled)

31. (Previously Presented) A method of enhancing the intrinsic enzymatic activity of an enzyme formed from fermentation comprising:
  - (a) diluting an enzyme solution comprising glucoamylase with an aqueous solution by a factor of at least three to provide a diluted enzyme solution;
  - (b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;
  - (c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and

(d) removing the activated carbon to provide an enzyme solution of enhanced activity.

32. (Previously Presented) The method according to claim 31, wherein the weight ratio of enzyme to activated carbon is not greater than 25:1.
33. (Previously Presented) The method according to claim 31, wherein the weight ratio of enzyme to activated carbon is not greater than 15:1.
34. (Previously Presented) The method according to claim 31, wherein the enzyme activity of the diluted enzyme solution after treatment with activated carbon is at least statistically equivalent to the enzyme activity of the enzyme solution before dilution.
35. (Previously Presented) The method according to claim 31, wherein the activity of the enzyme solution is enhanced by at least 200%.
36. (Previously Presented) The method according to claim 31, wherein the enzyme solution is diluted with the aqueous solution by a factor of about 5:1 to 10:1 times.
37. (Canceled)
38. (Canceled)
39. (Previously Presented) The method according to claim 31, wherein the aqueous solution comprises an aqueous buffer.
40. (Previously Presented) The method according to claim 31, wherein the aqueous solution comprises water.

41. (Cancelled)
42. (Previously Presented) An enzyme solution having enhanced activity made by a method comprising:
- (a) diluting an enzyme solution comprising at least one of glucoamylase or amylase with an aqueous solution by a factor of at least three to provide a diluted enzyme solution;
  - (b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;
  - (c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and
  - (d) removing the activated carbon to provide an enzyme solution of enhanced activity.
43. (Previously Presented) The enzyme solution according to claim 42, wherein the enzyme is amylase.
44. (Previously Presented) The enzyme solution according to claim 42, wherein the enzyme is glucoamylase.
45. (Previously Presented) The enzyme solution according to claim 42, wherein the activity of the enzyme solution has been enhanced by at least 200%.
46. (Previously Presented) A method of enhancing the intrinsic enzymatic activity of an enzyme formed from fermentation comprising:
- (a) diluting an enzyme solution comprising amylase with an aqueous solution by a factor of at least three to provide a diluted enzyme solution;



- (b) if the enzyme solution contains cells, filtering the diluted enzyme solution to remove the cells;
  - (c) treating the diluted enzyme solution with activated carbon at an effective raw enzyme weight to activated carbon weight ratio of not greater than 50:1 and for a sufficient period of time to effect said enhancement; and
  - (d) removing the activated carbon to provide an enzyme solution of enhanced activity.
- 47. (Previously Presented) A method as defined in claim 46 wherein said aqueous solution comprises an aqueous buffer solution.
- 48. (Previously Presented) A method as defined in claim 46 wherein said aqueous solution comprises water.
- 49. (Previously Presented) A method as defined in claim 46 comprising passing said raw enzyme solution through a column containing an effective amount of said activated carbon.
- 50. (Previously Presented) A method as defined in claim 46 wherein said activated carbon is removed by a method selected from the group consisting of filtration and centrifugation.
- 51. (Previously Presented) A method as defined in claim 46 wherein said raw enzyme solution is diluted with water to provide a diluted raw enzyme solution.
- 52. (Previously Presented) A method as defined in claim 46 wherein said raw enzyme solution is diluted with an aqueous buffer solution to provide a buffered diluted raw enzyme solution.

53. (Previously Presented) A method as claimed in claim 46 wherein said ratio is not greater than 15.
54. (Previously Presented) A method as defined in claim 46 wherein said enzyme solution of enhanced activity has a spectrum selected from Far UV (CD) and UV visible spectra distinct from said raw enzyme solution.
55. (Previously Presented) A method as defined in claim 54 wherein said enzyme solution of enhanced activity shows a relative absorbance intensity lower than said raw enzyme solution, in the CD spectral range of 205-230nm.
56. (Previously Presented) A method as defined in claim 54 wherein said enzyme is alpha-amylase and said enzyme solution of enhanced activity has a Far UV (CD) spectrum minimum ellipticity shifted by at least 1nm, from the raw enzyme solution, in the range between 205-230 nm.
57. (Previously Presented) A method as defined in claim 46 wherein said enzyme solution of enhanced activity has a UV-visible spectrum maximum peak at least 30 nm lower than said raw enzyme solution.
58. (Previously Presented) A method as defined in claim 46 wherein said enzyme is alpha-amylase and said enzyme solution of enhanced activity has a maximum spectral absorption peak over the range 340 to 360 nm.
59. (Previously Presented) The method according to claim 46, wherein the weight ratio of enzyme to activated carbon is not greater than 25:1.
60. (Previously Presented) The method according to claim 46, wherein the weight ratio of enzyme to activated carbon is not greater than 15:1.

61. (Previously Presented) The method according to claim 46, wherein the enzyme activity of the diluted enzyme solution after treatment with activated carbon is at least statistically equivalent to the enzyme activity of the enzyme solution before dilution.
62. (Previously Presented) The method according to claim 46, wherein the activity of the enzyme solution is enhanced by at least 200%.
63. (Previously Presented) The method according to claim 46, wherein the enzyme solution is diluted with the aqueous solution by a factor of about 5:1 to 10:1 times.
64. (Previously Presented) The method according to claim 46, wherein the aqueous solution comprises an aqueous buffer.
65. (Cancelled)
66. (Previously Presented) The method according to claim 42, wherein a pH of the enzyme solution having enhanced activity is from 3 to 8.

1. Rule 132 Declaration of Dr. Bradley A. Saville dated 26 April 2007. Submitted with Appellant's Response filed 2 May 2007.
2. Rule 132 Declaration of Dr. Bradley A. Saville dated 13 March 2008. Submitted with Appellant's Response filed 17 March 2008. Attachments 1 and 2 showing the experience of Dr. Saville is provided in the 26 April 2007 Declaration.
3. Cornell lab manual for BIOBM330, which discusses strategies for protein purification.  
<http://instruct1.cit.cornell.edu/Courses/biobm330/protlab/Strategy.html>  
Submitted with Appellant's Response and cited on PTO Form SB08A filed 2 May 2007.
4. Instructional materials for the MATC Biotechnology program in Madison, WI discuss methods and goals for protein purification.  
[http://matcmadison.edu/biotech/resources/proteins/labManual/chapter\\_1.htm](http://matcmadison.edu/biotech/resources/proteins/labManual/chapter_1.htm)  
Submitted with Appellant's Response and cited on PTO Form SB08A filed 2 May 2007.
5. Wingfield et al. (Eur. J Biochem. 180.23-32 (1989)). Submitted with Appellant's Response and cited on PTO Form SB08A filed 2 May 2007.
6. Mackay et al. (Fundamental and Applied Toxicology, vol 30, pp23 – 30, 1996). Submitted with Appellant's Response and cited on PTO Form SB08A filed 2 May 2007.
7. Pimenov et al. (Sep. Sci. Technol., 36(15), 3385-3394, 2001). Submitted with Appellant's Response and cited on PTO Form SB08A filed 2 May 2007.

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(x) Related Proceedings Appendix:

Not applicable.